THE BREEDING OF REEF ANIMALS

PART II. INVERTEBRATES OTHER THAN CORALS

BY

ANNE STEPHENSON,

Department of Zoology, University of Cape Town

WITH FOURTEEN TEXT-FIGURES AND FOUR TABLES

CONTENTS.

PREFACE.

The description of the breeding of corals, by Sheina M. Marshall and T. A. Stephenson, is published in Vol. III, No. 8 of these reports. The present work, on the breeding of invertebrates other than corals, was carried out by the author, with the assistance, during the latter part of the period, of E. A. Fraser, who took over the investigations of *Cypræa annulus* from 13th March to 15th June, 1929, and of *Myrionema amboinense* from 11th March onwards; and of S. M. Manton, who worked on *Thalamita stimpsoni* from 2nd April to 15th June, 1929, and on *Centrechinus setosus* from 2nd April to 14th June, 1929. Dr. Fraser has published an account of *Myrionema amboinense* in report No. 4 of this volume. Other members of the expedition also constantly helped throughout the year in the collection of both material and spawn. To all of them I extend my most hearty thanks; and also to T. A. Stephenson for guidance in the preparation of the report.

When selecting the animals on which to carry out the research, the aim was to choose representatives of the main invertebrate groups, with special reference to those which were most important on the reef. Unfortunately various circumstances considerably hampered the choice. For instance, the gastropod *Pterocera* and the star-fish *Linkia*, which would otherwise have been suitable, were so difficult to open up for the weekly examination of the gonads that it proved impracticable to use them. No worm or sponge

could be found which was both suitable and sufficiently plentiful; and prawns, though not uncommon, were difficult to secure. In arranging for a long period of weekly routine work on living animals, the tides played a very important part; during neaps certain animals would have been unavailable for long periods, and it was impossible to keep them artificially in the perfect condition which is essential for work on breeding.

The animals finally chosen were :

COELENTERATA.

Myrionema amboinense.

ECHINODERMATA.

Ophiothrix longipeda.
Tripneustes gratilla.
*Centrechinus (Diadema) setosus.**

MOLLUSCA.

*Acanthozostera gemmata.**
Cypræa annulus.
*Hippopus hippopus.**

CRUSTACEA.

Thalamita stimpsoni.

In addition to this, work was done by other members of the expedition on the breeding of the following animals :

Trochus niloticus. See F. W. Moorhouse, 1932.
Pinctada margaritifera. See A. G. Nicholls, 1931.

F. W. Moorhouse also worked on the gonads of several species of Bêche-de-Mer and of oysters, but his results are not yet available.

A NOTE ON THE NUMBER OF ANIMALS USED.

The numbers used in this paper are low (3655 animals were involved), which fact is due to several causes. It must be borne in mind that the reef itself was small, its major axis being only about one mile; the number of animals, therefore, of a large enough size for use in this investigation was not so great that there was an unlimited supply available for a year's work. This was particularly noteworthy in the case of *Tripneustes* and *Centrechinus*, the supply of which was running out by the end of the year. Then the animals which lived on the Thalamita flat,† *Ophiothrix* and *Thalamita*, were by no means unduly plentiful, and were not easy to catch. The supply of these two species and of *Acanthozostera* became very difficult during the period of bad day-tides in the summer. In addition to these restrictions, only a certain amount of time could be allowed for this work; the collection and examination of specimens, and the execution of experiments, is in any case a considerable undertaking.

Although, had it been possible, it would have been preferable to employ larger numbers, it is felt that too much stress can be laid on the necessity for large numbers in work of this description. The numbers actually used have, in fact, produced such consistent results that it is doubtful whether larger numbers would have affected the

* Animals marked with an asterisk are illustrated in Vol. III, No. 2 of these reports, Pls. XVIII, XX and XXI.

† For all references to habitats in this paper see Vol. III, No. 2, Text-fig. 2, of these reports.

conclusions apart from giving them a greater degree of precision. It may be noted in favour of these particular investigations that they deal with continuous observations for eleven months on animals living in a very restricted area. Further, the specimens used for any one species were collected as far as possible from a single habitat on the reef, and therefore belonged to a community subjected to no changes other than the ordinary seasonal ones.

DESCRIPTION OF THE RESULTS.

Myrionema amboinense.

Number of colonies examined, 478.
Weekly examination of 12 colonies, 11th August, 1928 to 3rd January, 1929; 29th January, 1929 to 15th June, 1929.
Average height of colonies, 1·5 cm.
Range of size, 0·7–3·8 cm.

In every month of the year, and in each week of each month (with three exceptions), some of the colonies examined bore definite gonophores. The proportion of fertile colonies varied from one month to another, but this variation means very little, since at first healthy-looking colonies were collected in preference to apparently poorly developed ones; but it was found later on that female gonophores occurred more commonly on colonies which had lost many of their polyps, and which therefore appeared less robust. It is clear, however, that from August 1928 to June 1929 some colonies of *Myrionema* were in a fertile condition. Whether the development of planulae occurred in all these months cannot be stated, but from Dr. Fraser's work in the months March to June, 1929, we know that planulae were being formed in March, April, May and June. An account of the details of development in this species has already been published by Dr. Fraser in Vol. III, No. 4 of these reports.

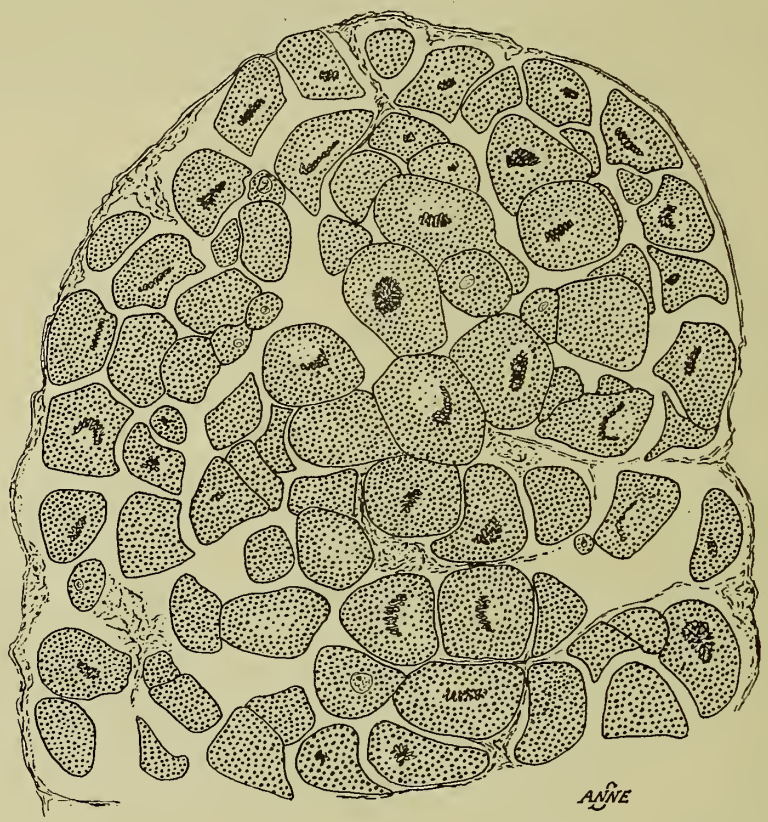
Ophiothrix longipeda.

Number of specimens examined, 388 (172♂, 180♀, 36 indeterminate).
Weekly examination of 12 specimens, 16th August, 1928 to 5th January, 1929; 30th January, 1929 to 25th April, 1929.
Further examinations 22nd and 29th May, 15th June, 1929.
Average diameter of disc 2·2 cm.
Range of size, 1·2–3·7 cm.

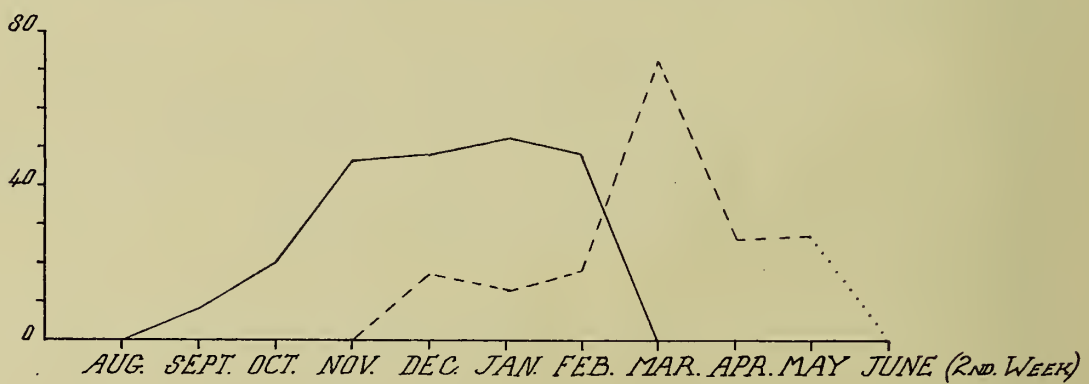
Ophiothrix longipeda was common on various parts of the reef; the collections were all made from under boulders on the Thalamita flat. The species is a large one, with a span of 2 ft. or more in full-grown specimens.

The Male Gonad.—When the animal was immature the testes were only visible as small white patches on the gut diverticula, and were often difficult to find; but when mature they were thick, spread well over the diverticula, and were a deep cream in colour, discharging a plentiful milky flow containing active sperms when cut. The sperms, when examined microscopically, never showed the intense activity of those of other animals examined, and their movements very rapidly subsided. Shortly after spawning had occurred the testes became small and collapsed.

The Female Gonad.—In the female the immature ovary was a brownish pink in colour, which changed to a dull but deep pink when the animal matured. The gonad then



Text-fig. 1.—*Ophiothrix longipeda.* Section of part of a ripe ovary. The nuclei are mostly in synizesis.



Text-fig. 2.—*Ophiothrix longipeda.* Graphs showing the percentage of mature and spent gonads (♂ and ♀) for each month. Continuous line, mature and nearly mature specimens; broken line, spent and resting specimens; dotted line represents probability only, since there was only one examination in June. The maximum number of mature individuals was reached in the summer months, the peak number of spent individuals following closely afterwards.

became of a very fluid consistency and the eggs (Text-fig. 1) were coloured reddish brown. In the ovary of a female which had spawned, there were found mature, immature, and disintegrating eggs, suggesting that another spawning might occur later.

Results of Routine Work.—The examination of specimens in August 1928, showed that they were immature ; in some cases it was difficult to determine the sex of the animal. By the end of September this difficulty had ceased, and a month later the definite activity of the sperms in the male, and the colour and other signs in the female, showed that the animals were maturing. By the end of November they seemed mature ; the males contained numerous active sperms, and the females plentiful mature eggs, the gonads in both sexes being large and extensive. During December and January the gonads mostly seemed mature, and occasionally specimens appeared which seemed to be spent or partially so. In February spawning was actually observed in two cases ; on the 6th one female shed its eggs during dissection ; another spawned spontaneously in the aquarium on the night of the 11th. Towards the end of this month the gonads had diminished in size and the colour of the ovary was fading. This continued throughout March and into April, when many specimens could not be sexually determined at all. At the end of May there were signs of returning activity. Numerous immature eggs were then discovered, and a very slight flow began. In the batch examined in June (only one collection was possible) these signs were continued, but the animals were still far from mature.

Conclusion.—From these data it seems clear that the spawning of *Ophiothrix longipeda* occurred during the summer months, December to February ; that there was only one spawning period in the year, followed by a resting stage in March and April ; and that maturing began again from May onwards. These points are well brought out in the graphs reproduced in Text-fig. 2.

Tripneustes gratilla.

Number of specimens used, 581.
Number examined in routine work, 363 (158♂ and 205♀).
Number used for experiments in April, May and June, 1929, 218.
Weekly examinations of 6 or 12, 7th August, 1928 to 3rd January, 1929 ; 29th January, 1929 to 25th April, 1929.
Further examinations on 23rd and 28th May, and 15th June, 1929.
Average diameter of animal, 8·8 cm.
Range of size, 6·8–10·8 cm.

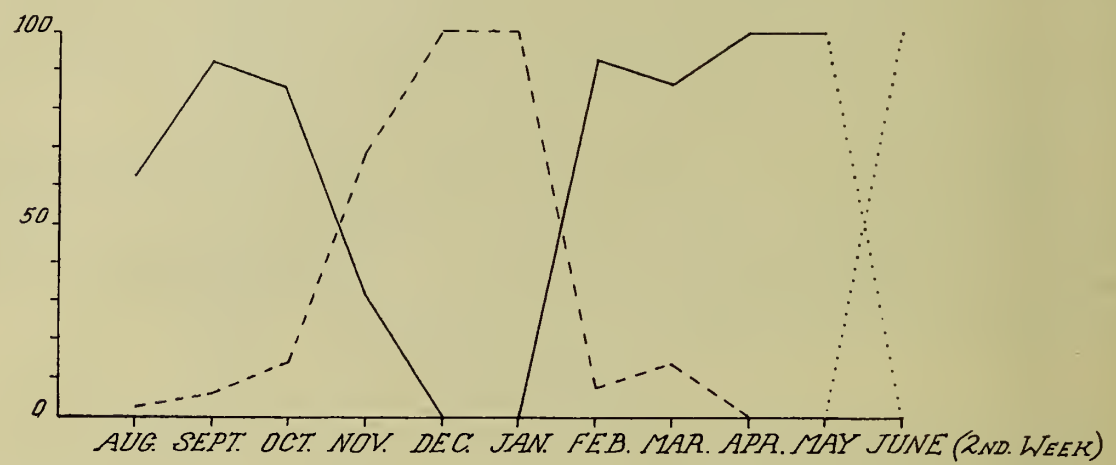
Of the two sea-urchins used in these experiments, *Tripneustes gratilla* gave the more satisfactory results. It was fairly common in certain parts of the reef, and was collected either from the Western Moat, or around the spit to which it gives its name. The species is a large Echinus-like form.

The Male Gonad.—When ripe the testes are large and plump, and usually cream in colour ; cutting produces a plentiful milky flow of myriads of extremely active sperms. This activity dies down fairly soon after spawning, and a spent gonad gives practically no flow when cut, the whole organ having a peculiar granular watery appearance. In immature and spent specimens the gonad has often a greenish tinge, and is shrunken in size.

The Female Gonad.—This has the same granular appearance when spent as that of the male, and all flow ceases, thus making the judging of its condition fairly easy. The

immature ovary is of a dark greenish colour ; on reaching maturity it is usually of a bright orange, and the yellow eggs pour out freely. The diameter of a ripe egg is about 90μ, and the nucleus is clearly visible.

Results of Routine Work.—When the observations were begun in August the specimens appeared to be maturing ; in September and October most specimens seemed mature. By the end of November there was a marked change in the appearance of the gonad in both sexes. The gonads had a granular appearance, no flow occurred when they were cut, eggs were disintegrating, and sperms few and inactive. However, the gonads were still thick and extensive. By the end of January the specimens seemed to be maturing again, and during March, April and May the majority of them appeared to be mature. The one examination in June showed specimens with all the signs of being spent, with the additional one that the gonads had decreased in size and were small and inextensive.



Text-fig. 3.—*Tripneustes gratilla.* Graphs showing the percentage of mature and spent gonads (♂ and ♀) for each month. Continuous line, mature and nearly mature specimens ; broken line spent and resting specimens ; dotted line represents probability only, since there was only one examination in June. The graphs indicate two periods of maturity with a quiescent period between.

Fertilization Experiments.—Attempts at artificial fertilization were frequently made during the weekly examinations, but apart from the fact that in reasonable circumstances (*i. e.* when the temperature was moderate), they agreed with the results obtained from the routine observations, they are not of any real value, as during the hot months the conditions made successful artificial fertilizations impossible. Three spawnings, however, were actually observed in the aquarium* ; these occurred on the nights of 24th February, 24th April and 9th May, 1929.

A further series of experiments was carried out by S. M. Manton. Specimens were placed upside down on a board overnight, and a record made of the number which liberated eggs or sperm fluid under these conditions.† These experiments began on 24th April and ceased on 20th June, 1929 ; 218 animals were used and 95 spawned. Details are given in Table I.

* Specimens placed in the aquarium were treated beforehand as in the case of *Centrechinus*, p. 254.

† This method was employed by H. Munro Fox in studying the breeding of sea-urchins in the Red Sea (1924, p. 529).

Conclusion.—From a consideration of the results of the weekly observations, it would seem that in *Tripneustes gratilla* there are two resting and two active periods during the year. In the year in question the first activity occurred between September and November, followed in December and January by a more or less quiescent period; from March to May there was another burst of activity, which died down to another and more complete period of rest. This is expressed graphically in Text-fig. 3.

Table I.—*Experiments on Board.*

Date. 1929.	Number of animals used.	Number which spawned.	
		♂	♀
April 24	6	3	1
,, 25	6	2	1
,, 26	7	1	4
May 9	12	2	3
,, 10	8	4	
,, 11	12	6	1
,, 12	12	8	2
,, 13	12	3	2
,, 14	6	1	1
,, 15	6		
,, 17	12	5	1
,, 20	11	4	1
,, 22	12	4	
,, 24	12	6	1
,, 27	12	8	3
,, 29	12	4	3
,, 31	12	2	2
June 3	12	3	2
,, 14	12		
,, 17	12		
,, 20	12	1	
	218	67	28

Centrechinus setosus.

Number of specimens used, 429.
Number examined in routine work, 350 (157♀, 76♂, and 117 indeterminate).
Number used for experiments, 79.
Board experiments on 24th, 25th and 26th April, 9th and 10th May, 1929.
Aquarium observations on 26th December, 1928; 26th January, 23rd and 24th February. 25th March, 24th April, 9th and 10th May, 1929.
Weekly examinations of 6 or 12, 7th August to 31st December, 1928; 31st January to 29th May, 1929.
Further examination on June 14th, 1929.
Average diameter of animal, 6·4 cm. (spines not included).
Range of size, 3·0–8·4 cm.

The sea-urchin *Centrechinus setosus* was dealt with in exactly the same way as *Tripneustes gratilla*. It was fairly plentiful in some parts of the reef, and was collected from the North-East and Western Moats, and around Tripneustes Spit. Apart from the regular weekly examinations of gonads, a number of specimens were placed on a board upside

down, overnight, as in the case of *Tripneustes*, by S. M. Manton. Further, on several occasions specimens were put in a cage on the reef, in which they could not feed, for two nights and days, in order to get rid of their intestinal content ; they were then transferred to the aquarium for observation, in the hope that they would spawn there.

The Gonads.—During the whole experiment the gonads in both sexes, which varied in colour from cream to gold, were usually thick and extensive, with no marked variation in size throughout the year. In this animal the number of records of " sex indeterminable " is very high, and probably many of these cases may have been immature males. The females were easier to identify, although the ova were often very small and embedded in tissue.

Results.—When the observations began in August 1928, a high percentage of specimens were difficult to determine as to sex, and the flow when the gonad was cut was either negligible or absent. The eggs, when present, were small and immature and deeply embedded in tissue. All these signs of immaturity slowly but definitely decreased as the year went on, and by November it was found that the sex of the animal could almost always be discovered easily. The flow in males was often plentiful and sometimes milky; myriads of active sperms were found; in females the eggs were larger, and freed themselves easily from the tissue. On 26th December, 1928, the only record of actual spawning was observed, when one specimen discharged a jet of sperm as it was being transferred from a collecting bucket into the aquarium. At about this time, also, isolated cases occurred of apparently spent or partially spent females, the gonads containing ova which seemed to be disintegrating. It was also noted that there seemed to be a slight fluctuation of ripeness in any one month, from August 1928 to March 1929. By March 1929, a definite decline in activity appeared to have set in, and in May as many as 50% of a batch of specimens would be indeterminable as to sex. Apart from the one case of spawning in the aquarium, neither keeping specimens in the aquarium nor inverting them on a board at night gave any positive result at all.

Conclusion.—Although the indications outlined above are somewhat unsatisfactory, they do point to a period of activity during the summer months, and to a quiescent period during the colder part of the year. It is curious that this species, in which Fox (1924) was able to demonstrate lunar periodicity in the Red Sea, should have given no certain sign of such periodicity at Low Isles. There is, however, no suggestion that periodicity was actually absent (note the fluctuation mentioned above within any single month) ; it might well have been possible to substantiate it with a fuller series of observations. In the Red Sea the species spawns for several months preceding and sometimes including September (*i. e.* in the warmer months as at Low Isles).

Acanthozostera gemmata.

Number of specimens used, 474.
Number examined in routine work, 335 (202♂ and 133♀).
Number used for experiments, 139.
Weekly examinations of 12, 6th August to 19th December, 1928 ; 28th January to 19th February, 1929.
Further examinations of 12 per month in March, April, May and June, 1929.
Average length of animal, 10·6 cm.
Range of size, 4·5–16·0 cm.

Acanthozostera gemmata is a chiton common on the reef, and has the advantage of growing to a large size—to a length of 14 cm. or more. The specimens used for dissection were collected on the Boulder Tract and on the beach Sandstone, C1. In the daytime the animal remains motionless in the crevices of the rocks and underneath and on the shady parts of boulders, but at night can be found creeping about with surprising speed over the exposed surfaces.

The Male Gonad.—The gonads were exposed by cutting off 4 or 5 of the 8 shell plates on the back with bone forceps. The colour of the male gonad is always red, but in immaturity it is of a dark shade, and when cut there is no flow, the gonad appearing a paler red inside ; as the gonad ripens the colour gradually becomes much brighter, and there is an extensive milky flow which, when the testis is quite mature, gives it a whitish appearance.

The Female Gonad.—The female gonad in immaturity is a very dark reddish brown, which changes to a deep blue black when the eggs are mature. In this latter condition it is so fluid that it is difficult to handle at all. The ova are black, and develop rays before they are discharged from the ovaries (see Text-figs. 4 and 5). They are easily visible to the naked eye, and are about 120μ in diameter. When the female gonad is spent it has a mottled brown appearance and becomes far more solid. In Text-fig. 4 is shown a section through a nearly mature female gonad. It will be noticed that this contains eggs in several stages of maturity. In the lower part of the figure are quite unripe eggs with small nuclei and no rays, while above there are well-developed free eggs with large nuclei and many rays. Text-fig. 5 shows a living mature rayed egg, drawn from a specimen which spawned 29th September, 1928.

Results of Routine Work.—The condition of the specimens in the weekly examinations gave the following interesting results :

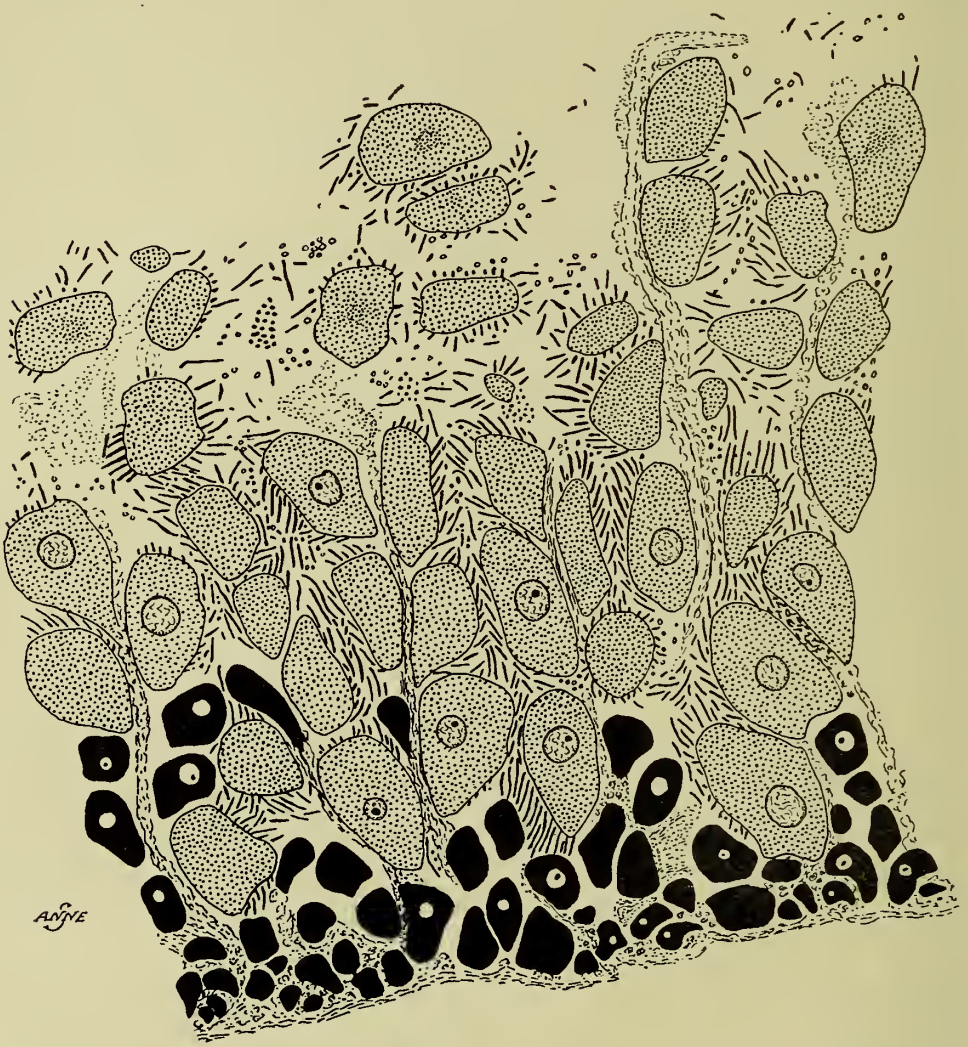
The earliest examination was of one odd specimen on 21st July, 1928, before the weekly examinations had begun. The gonad was so small and unripe that even the sex of the animal could not be determined. At the beginning of August, when the main observations were started, in several cases it was difficult to determine the sex of the animal, since neither males nor females showed any signs of maturity, and the female gonad was small and brown in colour. By the 27th of the month, however, several black ovaries were found, and there were also signs of sperm activity.

Through September the gonads ripened amazingly quickly, and at the end of the month mature specimens of both sexes were found. From then onwards until April 1929, a regular cycle appeared, of spent and maturing gonads in the first week of the month, maturing ones in the middle weeks, and mature ones at the end. The month of greatest activity seemed to be December. By the end of May, however, it seemed quite definitely to be the close of the spawning season, all gonads were small, and the flow was slight. The batch of specimens examined on 18th June showed no large or rayed eggs in females, and no milky flow or active sperms in any of the testes.

Experiments.—Apart from the observations just described, experiments were carried out with a view to obtaining supplementary information. Although these experiments could only be done on a small scale, the results were interesting.

Specimens were collected and put into glass jars of 3 litres capacity, one specimen being put into each jar. The mouths of the jars had covers firmly tied over them, made of several layers of butter muslin. This allowed some flow of water into them, but neither

animals nor eggs could escape. The jars were fixed in position by placing them in wooden tubs, which were sunk in the Western Moat, at such a depth that they were always half full of water. The tubs were firmly fastened to the reef and contained wooden partitions, enabling four jars to be fitted into each tub and left there, without any danger of their being broken by waves, etc. In this way jars were left out on the reef sixteen times with perfect safety. The experimental animals were put out on the reef in the afternoon of one day



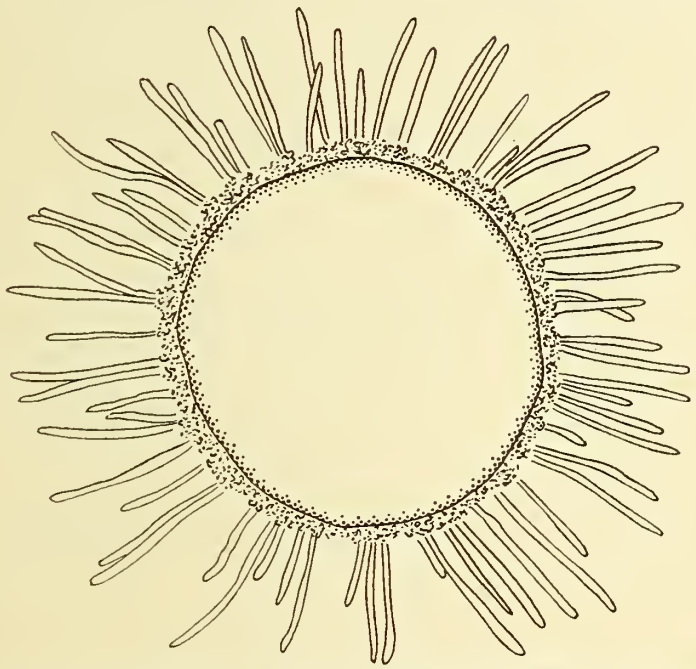
Text-fig. 4.—*Acanthozostera gemmata.* Section of part of a nearly ripe ovary. The young eggs which would mature in a subsequent month are black; the eggs nearly ready for extrusion are stippled. These latter eggs possess rays, which are cut at various angles.

and collected as soon as possible on the morning of the next, when samples of the water in the jars were examined for eggs and sperms.

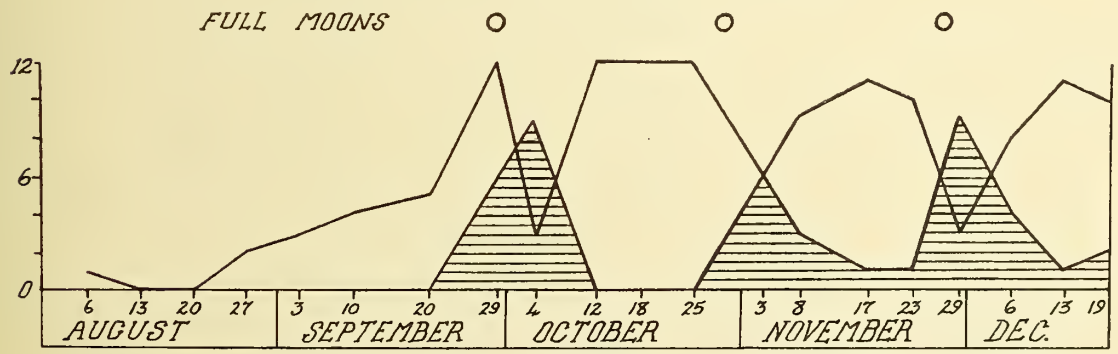
On six other occasions a different method was used as well as the one described above, *i. e.* a number of chitons were collected and kept in buckets of sea-water on the verandah for the night. This method proved less satisfactory than the other.

The dates of these experiments were carefully chosen. In eight cases the experiment was carried out on the night of full moon, and on all these occasions, except the last one (which was in May, after the breeding season), spawning occurred. The specimens which

spawned were always females, except in one instance, when sperms as well as ova were liberated in a bucket containing 6 chitons (27th December). It is possible that none of the males isolated in separate jars shed their sperms, because no female was present with them ; the presence of a female in the bucket perhaps provided a stimulus which caused the male to emit spermatozoa. In the case just quoted fertilization had occurred and early



TEXT-FIG. 5.—*Acanthozostera gemmata.* From a camera lucida drawing of a mature egg, extruded on 29th September, 1928. Diameter of egg 120μ.



TEXT-FIG. 6.—*Acanthozostera gemmata.* Graphs showing the number of mature and spent gonads (♂ and ♀) for each week, during part of the year. Shaded graph, spent or partially spent individuals ; unshaded graph, mature and maturing ones. A period of maturity occurs in each month at or before the time of full moon and a spent period follows each full moon.

segmentation stages were present. The remaining experiments took place on nights either immediately preceding or following the night of full moon, and on none of these occasions did any spawning occur.

CONCLUSIONS.—The above data show clearly that for several months the animal spawns and recovers every four weeks ; after this its activity weakens, the ovaries show no sign of a renewal of their stock of eggs, and gradually the animal enters into its resting

period. During the breeding season the flow of sperms and the fluidity of the female gonad vary markedly with a monthly rhythm; but in the quiescent period the colour and size of the gonads are notably different from their aspect during the months of activity.

It should next be noted that, during the breeding season, the part of each month during which the gonads are mature lies in all cases at or before the time of full moon (see Text-fig. 6); and the experiments show that specimens of the species do actually spawn on the night of full moon. There can therefore be no doubt that the species does spawn at about the time of full moon for several successive months in the year. The fact that in the experiments spawning occurred on the actual night of full moon *only* may, however, have been accidental, and need not be stressed.

TABLE II.—*Spawning Experiments on* Acanthozostera gemmata.

Date.	Condition of moon.	Method.	Number of chiton used.	Result.
1928:				
September 29	Full	Jars	?	Specimens spawned; number not noted.
October 29	,,	,,	6	2 ♀ spawned.
November 27	,,	,,	8	2 ♀ spawned.
December 25		,,	6	0
,, 27	Full	,,	6	2 ♀ spawned.
,, 27	,,	Buckets	6	1 ♂ spawned; unknown number of ♀ spawned.
,, 28		Jars	6	0
1929:				
January 26		Buckets	16	0
February 23		Jars	7	0
,, 23		Buckets	5	0
,, 24	Full	Jars	7	2 ♀ spawned.
,, 24	,,	Buckets	5	0
,, 25		Jars	7	0
March 25	Full	,,	6	1 ♀ spawned.
April 23		,,	8	0
,, 23		Buckets	6	0
,, 24	Full	Jars	7	1 ♀ spawned.
,, 24	,,	Buckets	5	0
,, 25		Jars	4	0
May 22		,,	6	0
,, 23	Full	,,	6	0
,, 24		,,	6	0
22 experiments.	Full moon on 8 occasions.	Jars 16 times. Buckets 6 times.	139 specimens.	More than 13 positive results.

Cyprœa annulus.

Number of specimens used, 490 (208♂, and 282♀).

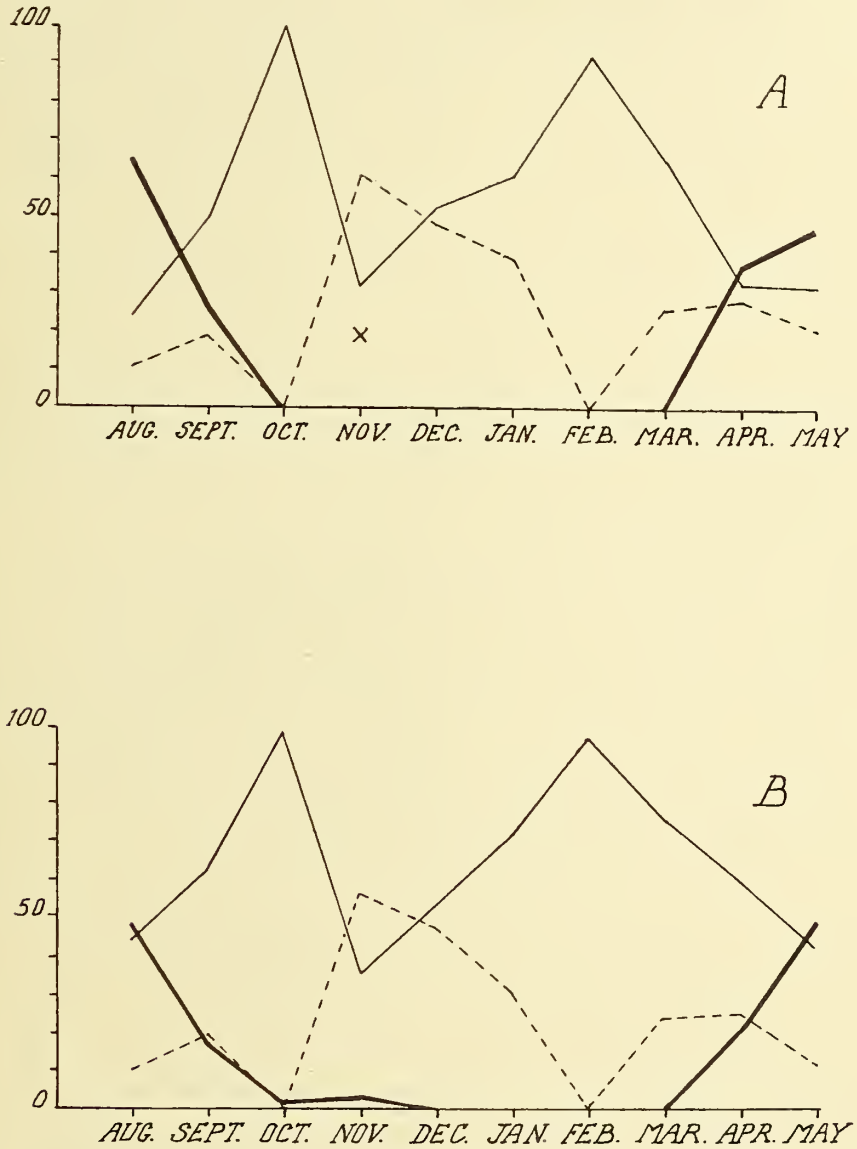
Weekly examination of 12, 9th August, 1928 to 2nd January, 1929; 29th January, 1929 to 30th May, 1929.

One examination on 15th June, 1929.

Average length of animal, 2·35 cm.

Range of size, 1·8–3·6 cm.

The animals were plentiful on many parts of the reef, and those examined were all collected either from *Hippopus* shells in the Mangrove park, or else from amongst the



TEXT-FIG. 7.—*Cypraea annulus*. The graphs in Fig. A apply to the gonads of females only; those in Fig. B to males and females taken together. Thick continuous line, immature; thin continuous line, mature or nearly so; broken line spent or partly spent. The percentage of specimens belonging to each category is shown for each month. Immature specimens are absent or nearly so in the warmer months, at which time spent and mature specimens are most numerous. The drop in mature specimens in November, coinciding with a peak among the spent ones, probably indicates approximately equal numbers of specimens ready to spawn or having just spawned. The cross in Fig. A indicates that in this month the greatest number of spawning individuals was found on the reef. The two series of graphs are given to show the close similarity between the results for one sex only, and for the two sexes taken together; this applies throughout to the cases recorded in this paper.

Thalassia on the flats around the anchorage. The shell, being very hard, was cracked in a vice, when the animal could easily be extracted.

THE MALE GONAD.—The testis was usually easily discernible, shading from a bright

orange to a brown or chocolate colour. The colour seemed to bear no relation to the ripeness or otherwise of the individual, although there might be a tendency for the testis to have a darker appearance towards the end of the spawning season. The sperms, of which there are two types, a normal and a vermiform, never showed the intense activity of those belonging to some other animals examined, nor was there such a plentiful or free flow of them when the testis was cut, even in specimens which were mature. There appeared to be no sudden dispersal of sperms and consequent immediate shrinkage in the size of the testis, as was observed in the case of *Ophiothrix*, for instance, but only a gradual decrease in the quantity and activity of the sperms, and therefore eventually in the size of the gonad. The exact condition of the animal was often difficult to determine in consequence.

The Female Gonad.—As in the male, the colour of the gonad did not indicate any particular stage in maturity; it was usually cream or yellow, and darkened a little towards the end of the spawning season. Probably several batches of eggs were laid before the animal became exhausted. This final stage was easy to determine, as the ovary then contained only disintegrating and immature eggs, and had become much smaller. At no stage did the eggs wash out very easily.

Results.—After careful consideration of the records, no outstanding breeding and resting periods can be distinguished, and certainly no lunar changes seem to affect the animals. A certain amount of fluctuation in the spawning activity, however, does undoubtedly occur. During August and September the number of immature specimens gradually decreased, and the number of specimens with well-developed gonads simultaneously increased. In October and November mature specimens were numerous, whilst the number of partially spent ones slowly increased. In November there appeared to be a higher percentage of partially spent than of mature ones, but by February ripe specimens once more were dominant. After February the number of mature animals steadily fell, and the number of spent and immature ones rose. By the beginning of May spent specimens were rare, and immature ones commonest. Unfortunately there is only one record in June, when the specimens examined were immature.

Conclusions.—In spite of the difficulty of interpreting the records, there seems to be little doubt that *Cypraea annulus* had a period of considerable activity during the summer months, possibly lasting from October until the end of February. There seemed to be a less active period during May and June, but possibly no complete resting period. Probably one specimen can spawn more than once in a year. The results of the routine observations on gonads are represented graphically in Text-fig. 7.

Apart from the observations on gonads, *C. annulus* was found spawning on the reef by various members of the expedition; the records of such spawning all occur between October and March, and although there was no systematic hunting, the records verify the supposition that *Cyprœa* was spawning in those months. Animals which were spawning or had just spawned were found on 28 occasions.

Hippopus hippopus.

Number of specimens used, 392.

Weekly examinations of 12 or 6, 8th August, 1928 to 2nd January, 1929; 1st February, 1929 to 25th April, 1929.

Further examinations 23rd and 25th July, 1928; 21st and 28th May, 17th June, 1929.
Fifty specimens examined between 1st and 4th February, 1929.
Artificial fertilizations: 47 attempts made on 17 occasions, extending over 7 months, 14th August, 1928 to 14th February, 1929.
Average length of shell, 24·0 cm.
Range of size, 14·4–33·0 cm.

Hippopus hippopus was an excellent mollusc for experimental purposes. It was one of the commonest animals on the reef, and lay about on the flats in hundreds, unattached. The one disadvantage was the weight of the creatures, but two people with a wooden stretcher could collect a dozen specimens comfortably in two journeys.

The Gonads.—*Hippopus hippopus* is hermaphrodite, of the type in which male and female acini lie side by side throughout the whole extent of the gonad (Text-fig. 10). The gonad, when mature (Text-fig. 8), was large and extensive, and light cream in colour. On cutting it there was a copious milky flow, which contained myriads of extremely active sperms, and free eggs with plainly visible nuclei, very often presenting that even appearance as of a field of marbles, so characteristic of samples from mature gonads. The condition of the gonad when spent (Text-fig. 9) was very distinctive; it was much less in bulk, and the cream colour had changed to a dull yellow with opaque patches, giving a mottled granular appearance to the whole organ. It was of a soft liquid consistency, but when cut there was no flow or only a slight clear one, which contained a decreased number of more or less inactive sperms, and irregularly-shaped eggs, often lacking any visible nuclei. On the other hand, an immature gonad was found to be small and of an even cream colour; when cut there was no flow, but the gonad contained numerous immature eggs embedded in tissue, and sperms neither plentiful nor active—the normal condition for an immature gonad.

Results of Routine Work.—When the main observations were begun in August 1928, the gonads were in a half-ripe condition, but as the summer approached there were signs of increasing maturity. By November the general aspect of most of the specimens was that of maturity, and on 7th December appeared the first specimen which gave evidence of having spawned. The following week provided two more such examples, but then, unfortunately, owing to absence from Low Isles, the examinations ceased entirely from 2nd January to 1st February, 1929. When the work was resumed on the latter date, it was found that every specimen of the first batch examined seemed to have spawned. To verify this, between the 1st and 4th of February 50 animals were examined, 20 microscopically in the laboratory and 30 on the reef *in situ*. In this latter examination the animals were collected in an entirely haphazard fashion from the Sand and Thalamita flats. These clams were opened, the gonads inspected, and a note made of their condition, which was easy to determine in the case of spent individuals. Of the 30 specimens thus examined, 23 had a positive spent appearance, and 7 were apparently partially spent; of the other 20 examined in the laboratory, 19 seemed completely spent and 1 partially so. Of the whole 50, therefore, 42 were quite spent and 8 less completely exhausted.

For the remainder of February and March, the weekly examinations gave consistent results in which the majority of animals were spent or nearly so. By April, however, no more partially spent specimens appeared, the gonads were small and any flow had

ceased, and they were more solid in consistency. Through May all signs of past spawning disappeared, and the examination in June gave a uniform result of immature gonads.

Fertilization Experiments. — Forty-seven attempts were made at artificial fertilizations, on 17 different occasions, between 14th August, 1928, and 14th February, 1929. Eggs and sperms were taken either from different individuals or from the same ones, and placed in bowls of sea-water, usually filtered, for a number of hours, samples being taken from them at intervals and examined microscopically. In no case was a

Text-fig. 8.—*Hippopus hippopus*. Part of a section of a mature gonad, for comparison with the section of a spent gonad shown in Text-fig. 9.

completely normal result obtained, which was probably, to a large extent, due to the facts that during the early part of the period the eggs were not sufficiently mature, and later, when the eggs and sperms were in a riper condition, the weather became too hot for success in such work, in a place where there were no adequate means of keeping the finger-bowls cool.

Until the end of November, 1928, the majority of the tests produced early segmentation stages, and frequently quite a high percentage of morulæ ; on one occasion in November, embryos in some cases had developed cilia. But there were nearly always present obvious abnormalities, and often at quite an early stage all the eggs died. As the spawning period

approached, the fertilizations became unsuccessful because of the heat. The results of these experiments fit in with the conclusions outlined below.

Conclusions.—From these data we may conclude that probably *H. hippopus* was in an immature condition in the Australian winter (June and July), that it ripened during the months of August, September and October, and that during November, December and January the gonads were mature, or nearly so. Probably some spawning occurred from December to March, since spent specimens could be found in each of these months ;

Text-fig. 9.—For explanation see Text-fig. 8.

but the very high percentage of spent individuals found at the beginning of February suggests that the greater part of the spawning had occurred during January.

From 7th December to the end of the month the surface temperature in the Anchorage, in the afternoon, was always above 28·0 C., rising at its highest to 30·7° C. on the 24th. Probably spawning first began during this period. A second hot spell started on 12th January, and from that date until the 30th the afternoon temperature in the Anchorage was always above 29·0 C., rising at its highest to 31·98° C. on the 19th. In all probability the spawning of the majority of the clams took place during this second still hotter spell. This would be in line with the work of Orton, Nelson, and others who have shown that certain lamellibranch species spawn after the temperature has reached a certain definite level.

TEXT-FIG. 10.—*Hippopus hippopus.* Part of a section of a gonad, showing a group of male acini and a few ova.

Thalamita stimpsoni.

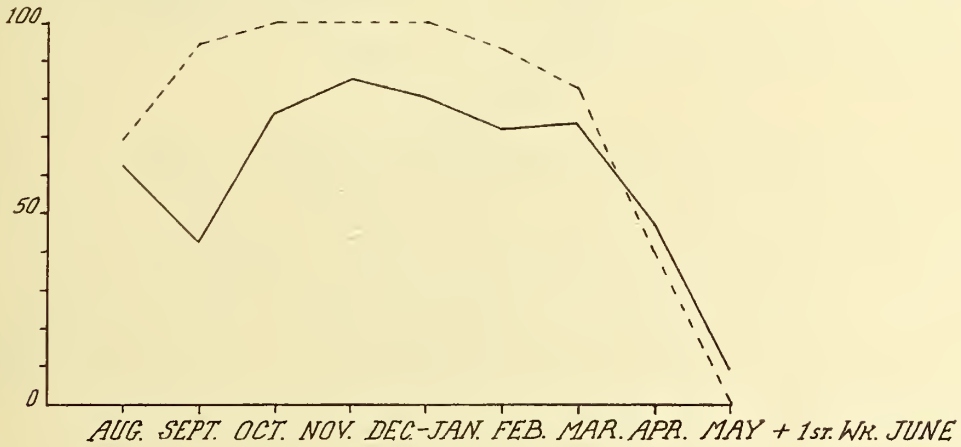
Number of specimens examined, 423 (211♂ and 212♀).
Weekly examinations of 12 specimens (approximately), 10th August, 1928 to 5th January, 1929 ; 6th February, 1929 to 15th June, 1929.
Average width of carapace, 5·0 cm.
Range of size, 3·2–7·0 cm.

This crab was fairly numerous amongst the rocks and boulders of the reef flat, especially in that part which is named after it, the Thalamita flat. As already mentioned, it was not easy to secure a regular supply of specimens, particularly when the tide did not entirely leave the flat, as at neap tides. This is the reason for some of the irregularities in the records of weekly examinations.

The animals were usually collected and examined on the same day; if, however, they had to be kept for a time, for instance when the tide made it only possible to collect them at night, they were stored in covered baskets in the sea. In addition to the examination of gonads, spawn carried by females was examined, and notes taken of the stages of development reached by the embryos.

The Male Gonad.—In the case of males it was found that nearly all the specimens contained formed spermatozoa ; but from October 1928 onwards an increasingly high percentage of the sperm occurred enclosed in spermatophores, filling the vas deferens. The testes increased in size during the first few months (August to October), but after January 1929, the development slowly became less and less ; the vas deferens, however, remained distended with spermatophores.

The Female Gonad.—At no time during the year was there a period in which females with empty ovaries were numerous ; but the condition of the eggs and the size of the gonad showed a definite variation in certain months. In August 1928, when the work began, usually all specimens contained thick and extensive ovaries with maturing eggs. By October there was a higher percentage of individuals with immature ova, and this continued throughout November ; the ovaries were still large and extensive. From December to March large ovaries and mature eggs again predominated. After this period,



Text-fig. 11.—*Thalamita stimpsoni*. Graphs showing (*a*) the percentage of females bearing embryos, in each month (continuous line) ; and (*b*) the percentage of males with large gonads (broken line). The first graph shows that berried females occurred in every month, but more commonly in the warmer months ; the second indicates more widespread activity in the male gonad in the warmer months than in the cooler ones. Occurrence of spermatophores in the male is not included here.

however, an increasing number of specimens showed thin and inextensive gonads (sometimes the ovaries could not be identified at all), containing variously mature or immature eggs. By the end of May nearly all specimens had small gonads containing immature eggs. The one examination in June showed a similar state of affairs.

Females were found carrying embryos from August through to March, the highest percentage of berried crabs being reached in November and December. A marked diminution in the numbers occurred in April, and in May the percentage of berried females is very low, coinciding with the presence of small gonads with immature eggs. Spent females with soft shells occurred intermittently throughout most of the year. The condition of the gonad in the females carrying embryos was somewhat variable ; but only in the months when berried females were scarce were the gonads of the majority in a relatively inactive condition.

Conclusions.—The fact that berried crabs were to be found in every month from August 1928 to June 1929 shows that at least some reproduction occurred during the greater part of the year, if not all of it. At the same time the percentage of females carrying embryos varied markedly, and when plotted for the period of observation shows a fairly regular curve (Text-fig. 11), the percentage in the warmer months being uniformly high. The cycle of changes in the ovaries of the females also indicated greater activity in the warmer months than in the others. The curve illustrating the percentage of males with large gonads (Text-fig. 11) tells the same story. It is true that the males continued to have spermatophores in the vas after the period of greatest activity in the testes was over* ; but since spermatophores in Crustacea are sometimes stored for a long time (it is well known that sperms contained in spermatophores may live for months or years carried by the female), their presence does not necessarily imply *recent* testis-activity on the part of the individual possessing them. It seems a fair conclusion that *Thalamita stimpsoni* spawns to a certain extent for most of the year, but that spawning occurs in a much higher proportion of the population in summer than in winter.

SPAWN COLLECTED ON THE REEF.

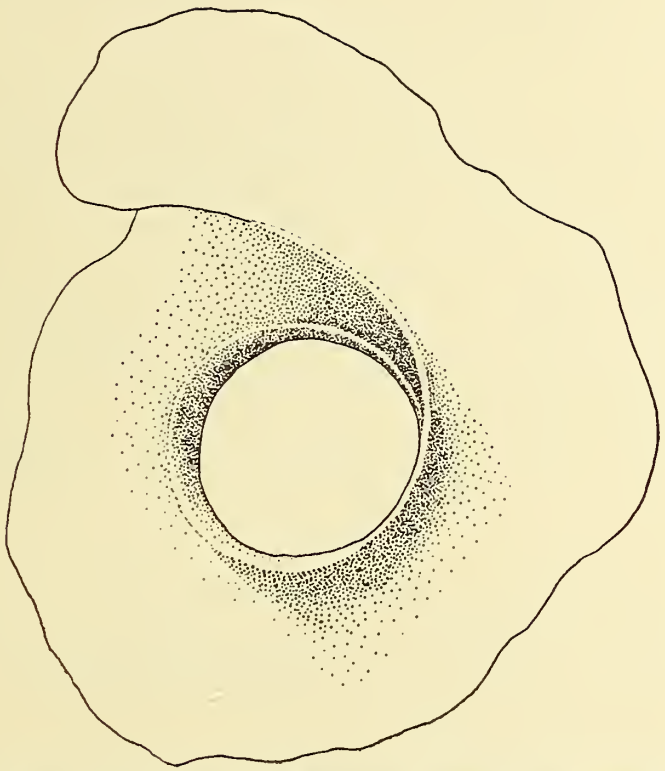
During the year a record was kept of spawn collected on the reef by any member of the expedition. In the case of each specimen the spawn was described and its condition noted. In some cases where the animal was found actually depositing the spawn it was possible to make a definite identification ; in other cases only an approximate determination was possible. This collection was not at all systematic, but its results are recorded, since they yield useful data.

The total number of specimens of spawn collected during the ten months, August 1928 to May 1929, was 400, with the addition of many specimens which were recorded simply as " numerous ". From the note at the foot of Table IV it will be seen that in each of these ten months at least 8 species of animals were found spawning on the reef. The number for some months was much higher—up to 21 species in October. The fluctuation in numbers from month to month means very little because of the casual nature of the collecting. The number of days in each month on which spawn was collected varied from 5 to 16. It is noteworthy that in every month one or more species of the genera *Natica* and *Uber* (Text-figs. 12, 13) was spawning, and sometimes the spawn-coils of these animals were extremely numerous, much more so than the table would indicate.

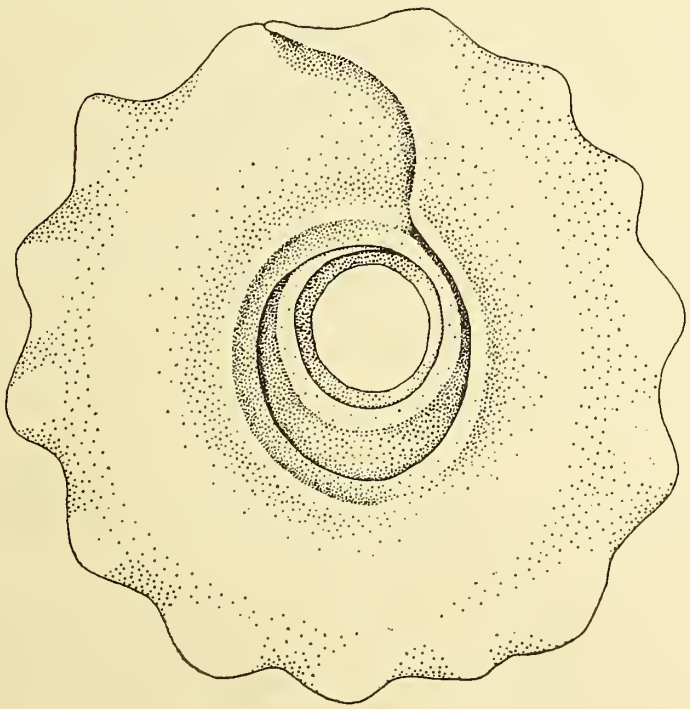
SUMMARY AND CONCLUSIONS.

In this summary, which includes the results described in Parts I and II of this paper, together with those of Moorhouse and Nicholls, a differentiation has been made between facts which are certainly known and those which seem probable, but in the latter case the degree of probability is so high in some cases as to amount almost to certainty.

* From a study of such literature as is available in South Africa, the only record of a probably similar case was found in Andrews, 1911, pp. 428–9.



TEXT-FIG. 12.—This figure and Text-fig. 13 illustrate two sandy coils containing egg-capsules, made by species of *Natica* (or *Uber*); they show the different pattern of coil formed by two distinct species.



TEXT-FIG. 13.—See Text-fig. 12.

During the period August 1928 to June 1929, the following observations were made on the breeding of animals at Low Isles :

COELENTERATA.

Myrionema amboinense.—This hydroid bore gonophores throughout the period ; it produced planulae from March to June, and perhaps in the other months also.

Favia doreyensis.—This coral probably spawned chiefly in December.

Porites haddoni.—This coral spawned continuously from January to July ; there appeared to be a falling off towards the end of this period.

Pocillipora bulbosa.—This coral spawned in nearly every month of the period ; it appeared to exhibit definite lunar periodicity, the spawning periods occurring at about the time of new moon from December to April, and about the time of full moon in July and August, with a transition period in May and June.

Pavona cactus.—A single record of spawning of a male colony of this coral was obtained in November.

ECHINODERMATA.

Ophiothrix longipeda.—This brittle star spawned in February ; its breeding period probably extended from December to February.

Tripneustes gratilla.—This urchin spawned in April, May and June. Almost certainly it had an earlier spawning period about September to November the year before, with a resting time during December and January.

Centrechinus setosus.—Spawning was observed in December ; the breeding period probably extended from about November to March ; lunar periodicity was suspected, but not demonstrated.

MOLLUSCA.

Acanthozostera gemmata.—This chiton spawned in the months September, October, November, December, February, March and April, at the time of full moon. The gonad samples confirmed the lunar periodicity observed in the actual spawning.

Cypraea annulus.—Spawning in this cowrie occurred in the months October, November, December, January and March. The breeding season probably extended from October to March.

Hippopus hippopus.—Most individuals of this clam probably spawned rather suddenly in January. It is likely that some breeding took place from December until March.

Trochus niloticus.—This gastropod spawned in March, June and July, and probably for a longer time than this (Moorhouse).

Pinctada margaritifera.—(The black-lip pearl-oyster.) According to Nicholls, this species spawned in November and May.

CRUSTACEA.

Thalamita stimpsoni.—This crab spawned throughout the period, but gave clear indications of a particularly active breeding season during the months October to March.

GENERAL.

From the collection of spawn on the reef it was ascertained that during each month of the period at least eight animal species were spawning ; in some months many more than this. The details are given in Tables III and IV, pp. 269, 270.

Table III.

Animal.	1928. August.*	Number of specimens.	September.	Number of specimens.	October.	Number of specimens.	November.	Number of specimens.	December.	Number of specimens.	1929. January.	Number of specimens.	February.	Number of specimens.	March.	Number of specimens.	April.	Number of specimens.	May.	Number of specimens.
GASTROPODA.																				
Cypraea annulus					25	1	8, 10, 23	19	31	3	27	2			4, 27	3				
,, *arabica*					23, 28	2					26	1	19	2						
,, *lynx*					27	1									21	1				
,, *vitellus*					23	1	22	1			4	1	6, 7	3						
,, sp.	24, 25	2			24	1	26	1												
Conus sp.							23	1			4, 5	2	21	1	23	1				
Iopas sertum					27, 28, 29	7														
Melo diadema			28	1	10, 25	2														
Natica and *Uber* (7 species)	24, 28, 29, 30	5	7	17	13, 16, 25	8	8, 10, 14, 19, 23	21	6, 17, 18, 26, 28, 31	13	4, 29, 30, 31	6	6, 7, 8, 11, 12, 13, 14, 20, 21, 22, 27	31	6, 8, 10, 11, 18, 22, 23, 26, 28, 31	36	3, 4, 6, 12, 16, 19, 20	59 N.	18, 28	4
Turbo concinnus					15, 25, 28	5	22	1												
Aplysia (?) (2 species)	24	1	8, 10	11	8	1	8, 11, 19, 22, 23, 27	7	6, 20, 23	3	4, 30	3	6, 18, 19, 20, 22	13	8, 31	3	4	3		
Hydatina sp.	27	1			25	1														
Nudibranch	23	1	7	1					31	1										
Opisthobranch (2 species)					8	1			26	1	24, 28	4	7, 13	8			8	1	26	1
Opisthobranch (?)	27, 29	2			8, 25	2													27	1
Gastropod (9 species)	30	1	6, 13	2	5, 25	2	3, 10, 22, 23	10 N.	14	1			7, 14	2	1, 10, 18, 22, 28	7	6, 12, 14	3S.	2, 28	3
Gastropod (?) (4 species)			13	1							27	1	17	1	18, 31	2	8, 26	1S.	18, 27	1S.
CRUSTACEA.																				
Cryptochirus					15	2							6	1						
Hermit crab	26, 28	5													31	3				
POLYCHAETA.																				
Polychaets			4, 8	N.	3	N.							28	N.						

* The numbers listed under the months refer to the dates in each month on which the species in question was found. The number of specimens refers to specimens of spawn. N., numerous; S., several.

TABLE IV.

Animal.	Dates.	Number of specimens.
GASTROPODA :		
Cypraea moneta	November 10	1
,, ,, (golden)	April 26	1
,, *staphylea*	October 23	1
Conus textile	November 23	1
Magilus	February 2	1
Aplysia	October 29	3
Aplysiomorph	November 10	1
Dolabella (?)	August 30	1
Hydatina amplustre	November 6	2
Philine	August 29	1
Pleurobranch	October 25	1
CEPHALOPODA :		
Cephalopod	March 31	1
CRUSTACEA :		
Caphyra levis	August 27	2
Crab (?)	August 28	1
Gonodactylus glabrous	November 5	1
Panulirus	December 18	1
Trapezia cymodoce	January 8	1

The number of species spawning in each month was as follows : August, 14 ; September, 8 ; October, 21 ; November, 18 ; December, 9 ; January, 11 ; February, 15 ; March, 14 ; April, 11 ; May, 8.

The following general conclusions may be drawn from the above data.

(1) Breeding of animals was by no means confined to any one part of the year ; it occurred in every month, and therefore in winter as well as in summer.

(2) Examples were found of the following types of breeding :

(*a*) A single breeding period in the year not lasting the whole year round (*Favia*, *Ophiothrix*, *Hippopus*, *Cypraea*, *Trochus*, etc.).

(*b*) Continuous breeding throughout the year, but more active in one part of the year than during the remainder (*Thalamita*, and possibly *Myrionema* and *Porites*).

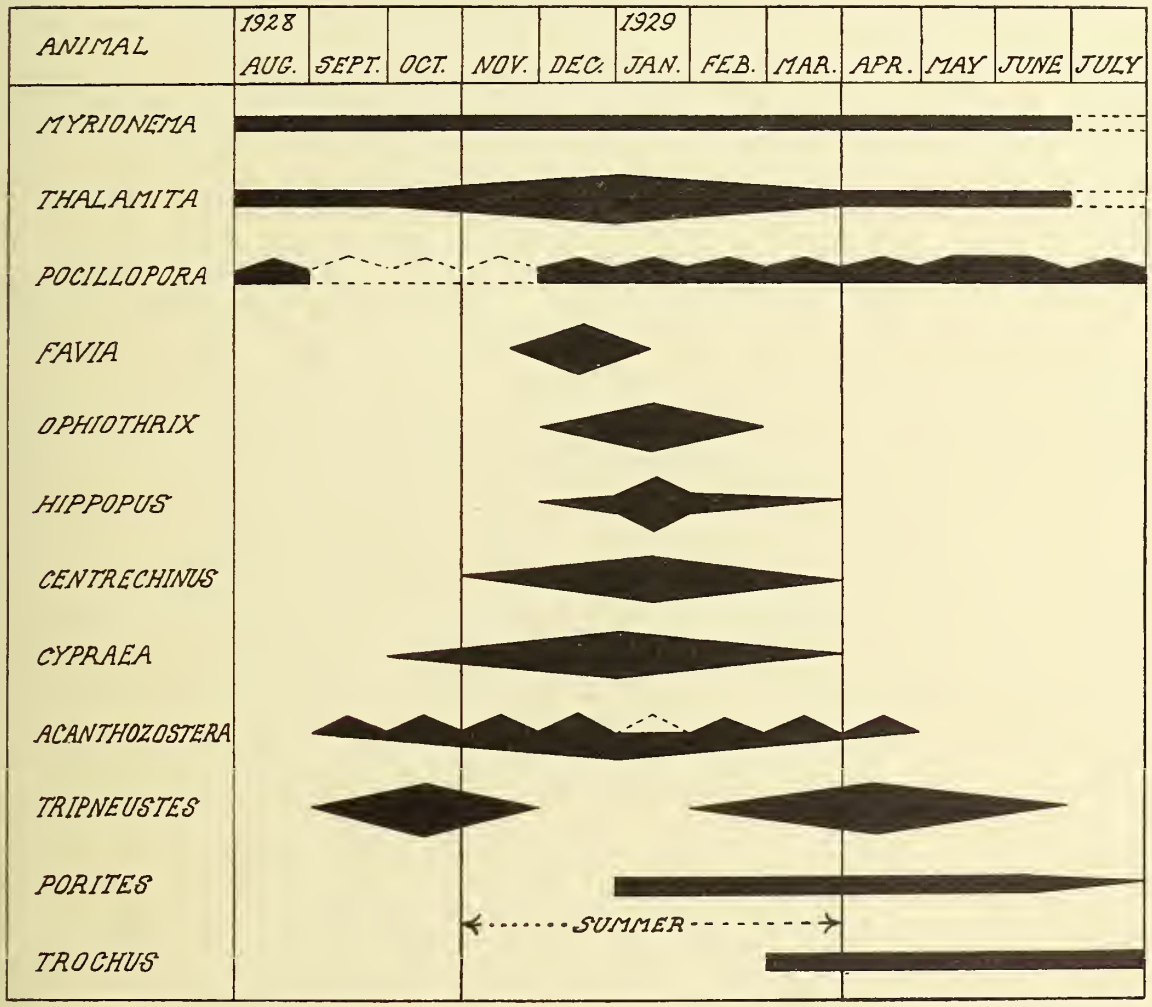
(*c*) Discontinuous breeding, occurring in relation to lunar phases, during a longer or shorter portion of the year (*Pocillopora*, *Acanthozostera* and possibly *Centrechinus*).

(*d*) Two spawning periods in the year, with a quiescent phase between them (*Tripneustes* and probably *Pinctada*).

(3) Although some species were breeding at all times of the year, in 7 out of the 13 species specially studied, the breeding period was either contained in or had its maximum in some part of the summer. This is well brought out by the diagram reproduced as Text-fig. 14, in which the breeding seasons of these species are graphically represented.

(4) Summarizing the whole work, it may be stated that on the Low Isles Reef during the period investigated, spawning of animals was going on the whole year round, but that if a census of all species could be made, a majority of them would be found spawning either exclusively or most actively in the warmer months. Probably Low Isles is very typical of tropical marine areas in this respect.

An investigation of wider scope than the present would no doubt add corrections of detail to the conclusions here set out. Especially, the actual breeding-season of any one species would probably fluctuate considerably from one year to another, in the same place, not to mention its varying from one district to another ; but the broad conclusions here stated are well founded, and can be amplified as new information comes to hand.



TEXT-FIG. 14.—Diagram indicating the probable spawning periods of the species specially studied at Low Isles, as described in the summary. Broken lines show probabilities where there are no data. Periodicity is indicated by a succession of peaks (the peaks not adjusted to moon phases). In the cases of *Thalamita* and *Hippopus* the wider part of the figure implies increased breeding activity ; in the plain diamond-shaped figures the shape is purely conventional, and indicates simply the *duration* of the breeding period ; *i.e.* the widest part does not imply the most active part of the period. In the case of *Porites* and *Trochus* the figure starts abruptly, since the beginning of the period is unknown.

This paper might be made an opportunity for discussing the general question of lunar periodicity ; especially since two new records are made of the occurrence of this phenomenon, and one of them appears to be of a novel character (*Pocillopora*). Since, however, we have at present no new theory to suggest which will account for all the facts,

and as these facts have been summarized elsewhere, no further comment will be made. Recent literature on periodicity is listed in Part I.

When the plankton-work of the expedition is published, further information will become available. No reference is made to plankton in this paper since very few of the results have as yet been printed.

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